

TS 5

Innovations in the Factor VIII:C Inhibitor Assay

Bert Verbruggen

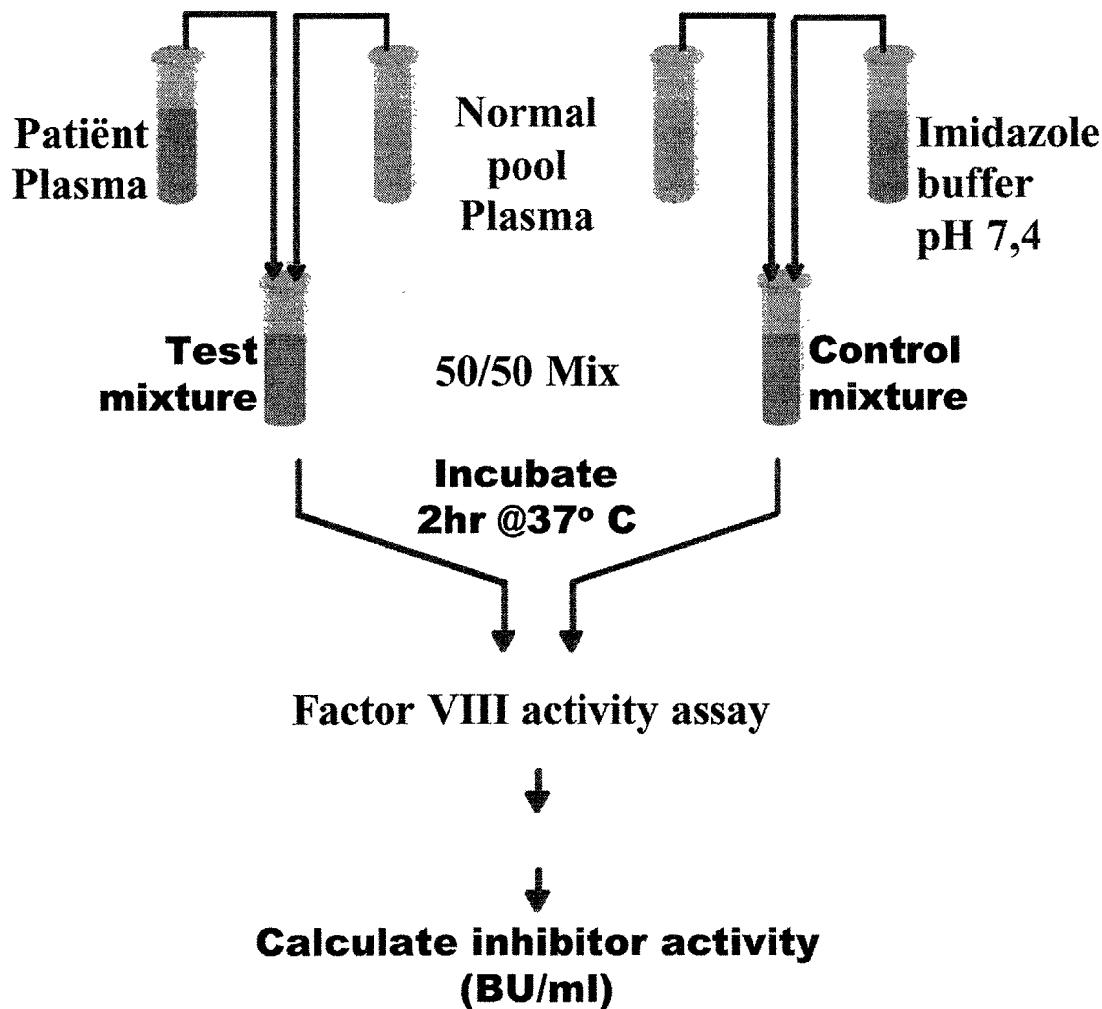
Workshop on Factor VIII inhibitors, November 21, 2003

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Innovations in the Factor VIII:C Inhibitor Assay

- Specificity
- Accuracy
- Precision/Inter-Laboratory Variation
- Sensitivity

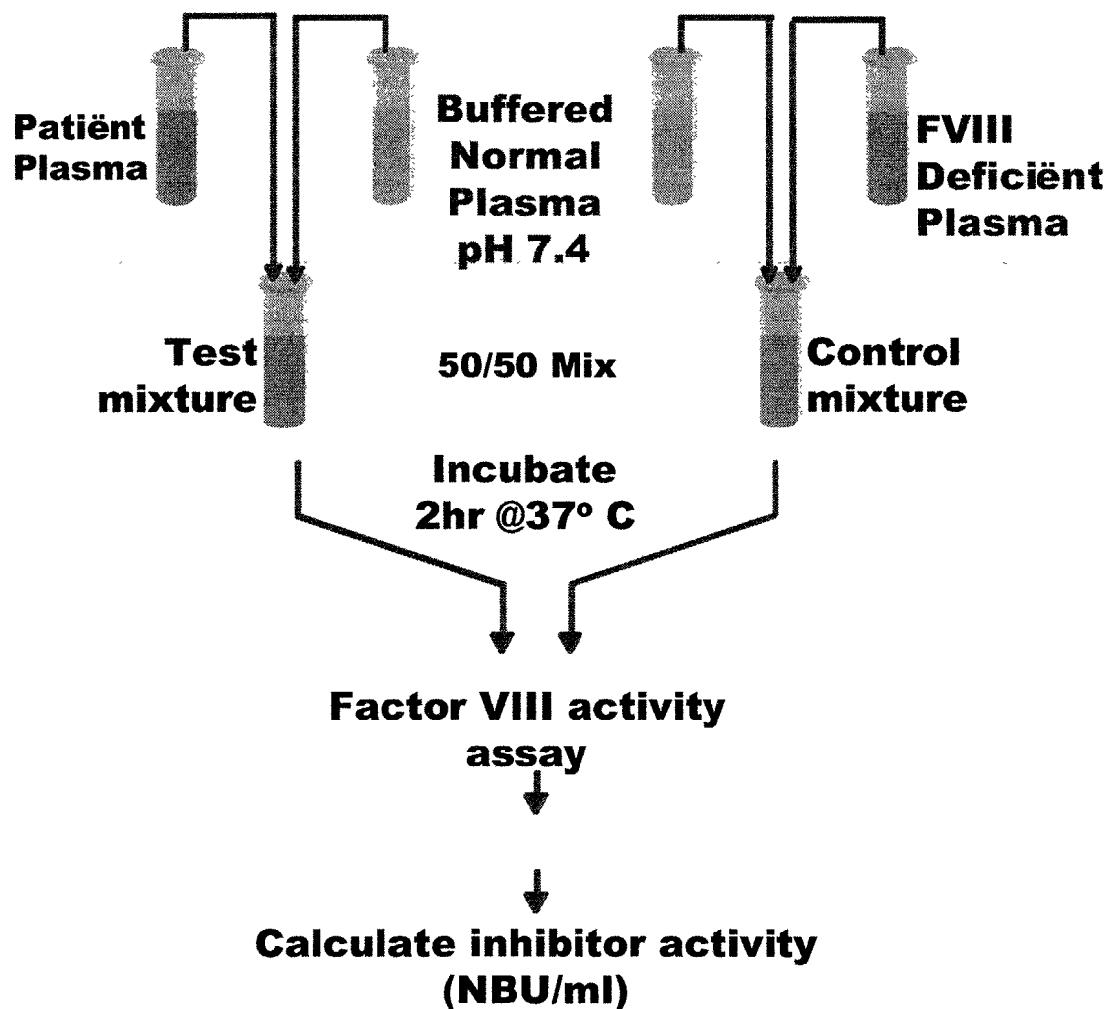
CLASSICAL BETHESDA ASSAY



Specificity

Specificity is the ability to obtain normal results in
normal situation

NIJMEGEN-BETHESDA ASSAY



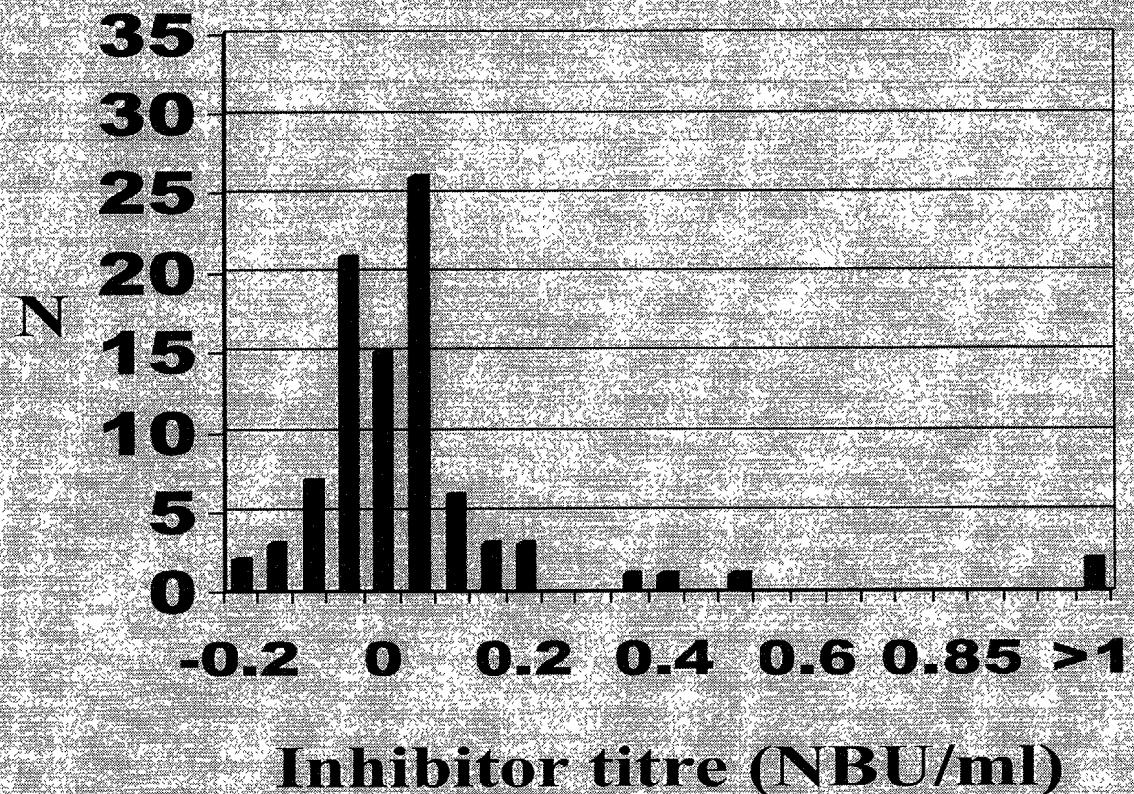
Nijmegen versus Bethesda Assay*

Results (BU)	CB	NB
0	792	824
> 0 - < 0.5	38	18
≥ 0.5	47	35

CB = Classical Bethesda assay, NB = Nijmegen modified Bethesda assay

* Giles et al. T&H 1998; 79:872-5

Nijmegen Experience



Specificity (conclusions)

False positive inhibitor results have been eliminated in the Nijmegen-Bethesda assay by:

- buffering of Normal Pool Plasma, and
- replacing Imidazole buffer as control by F.VIII deficient plasma.

Accuracy

Accuracy is a measure of agreement between the estimates of a value and the true value

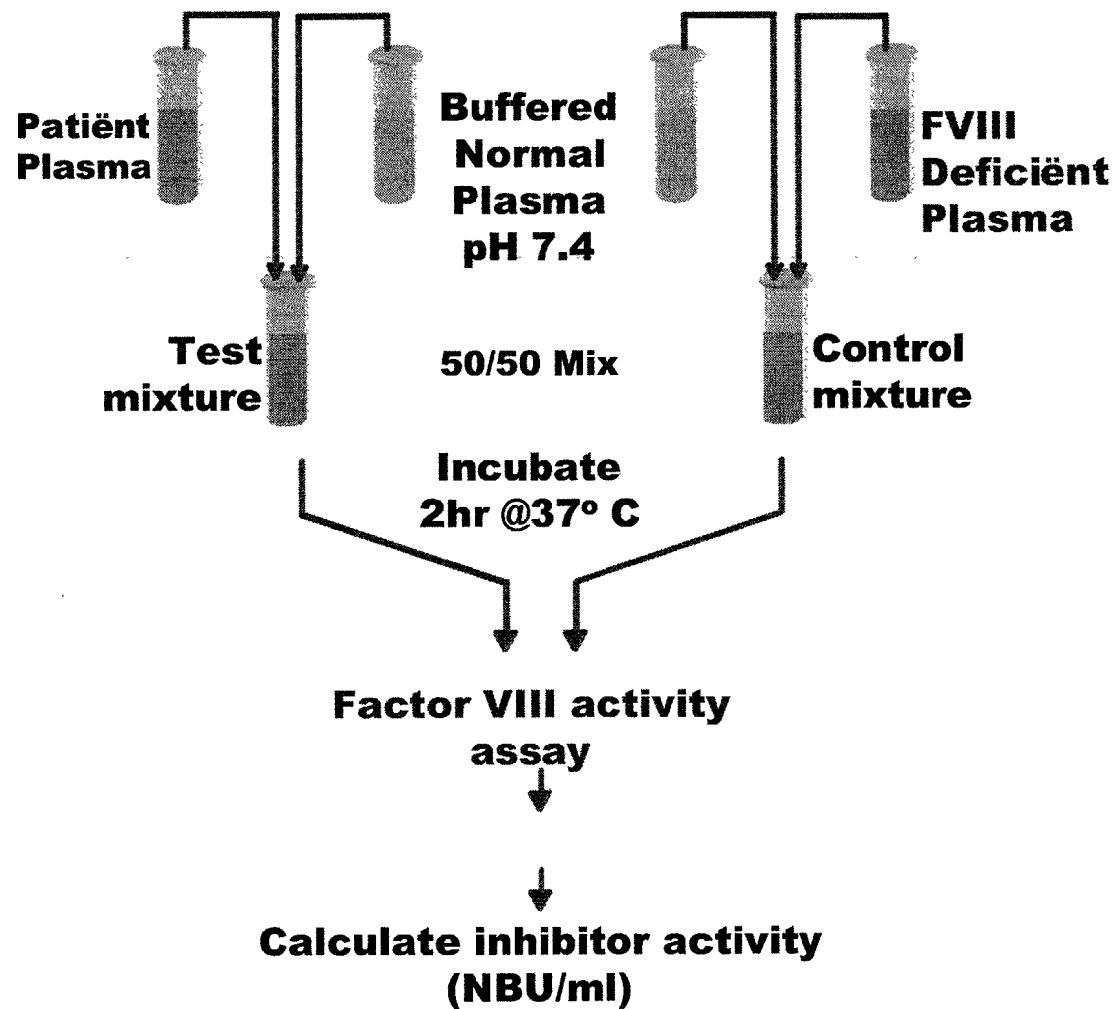
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Two theoretically important items:

- F.VIII activity in Normal Pool Plasma used as F.VIII source in the incubation mixtures
- Variations in F.VIII:C activity assay

NIJMEGEN-BETHESDA ASSAY



Influence of Type of F.VIII Deficient Plasma on Inhibitor Titre

Type of control plasma	Type of substrate plasma in factor VIII assay			
	Chemical depleted	Immuno depleted-1	Immuno depleted-2	Cong deficient
Chemical depleted	0.94	1.66	1.19	1.66
Immuno depleted-1	0.97	0.58	0.80	1.10
Immuno depleted-2	0.97	0.79	0.77	1.19
Congenital deficient	0.99	0.87	0.93	1.16

ACCURACY

The accuracy of the Nijmegen Bethesda assay is influenced by type of F.VIII deficient plasma used

Inter-Laboratory Variation

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Inter-Laboratory Variation

Sample	Nijmegen Assay(14) mean titre (CV)	Bethesda Assay(4) mean titre (CV)
1	13.7 (36 %)	12.3 (59 %)
2	4.5 (28 %)	4.4 (26 %)
3	13.8 (33 %)	10.6 (28 %)
4	31.6 (25 %)	31.0 (34 %)
5	32.1 (29 %)	33.4 (32 %)
6	34.2 (31 %)	38.0 (31 %)

SSC Collaborative study, Steven Kitchen, 2001/2002

Sensitivity (1)

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**F.VIII:C inhibitor positive
by “golden standard”**

**Inhibitor test positive
(\geq CUTOFF VALUE)**

a

**Inhibitor test negative
($<$ CUTOFF VALUE)**

b

Sensitivity

$a/(a+b)$

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NO

Sensitivity (3)

- F.VIII kinetics after replacement and bleeding status are “golden standards” but not “18 carat”

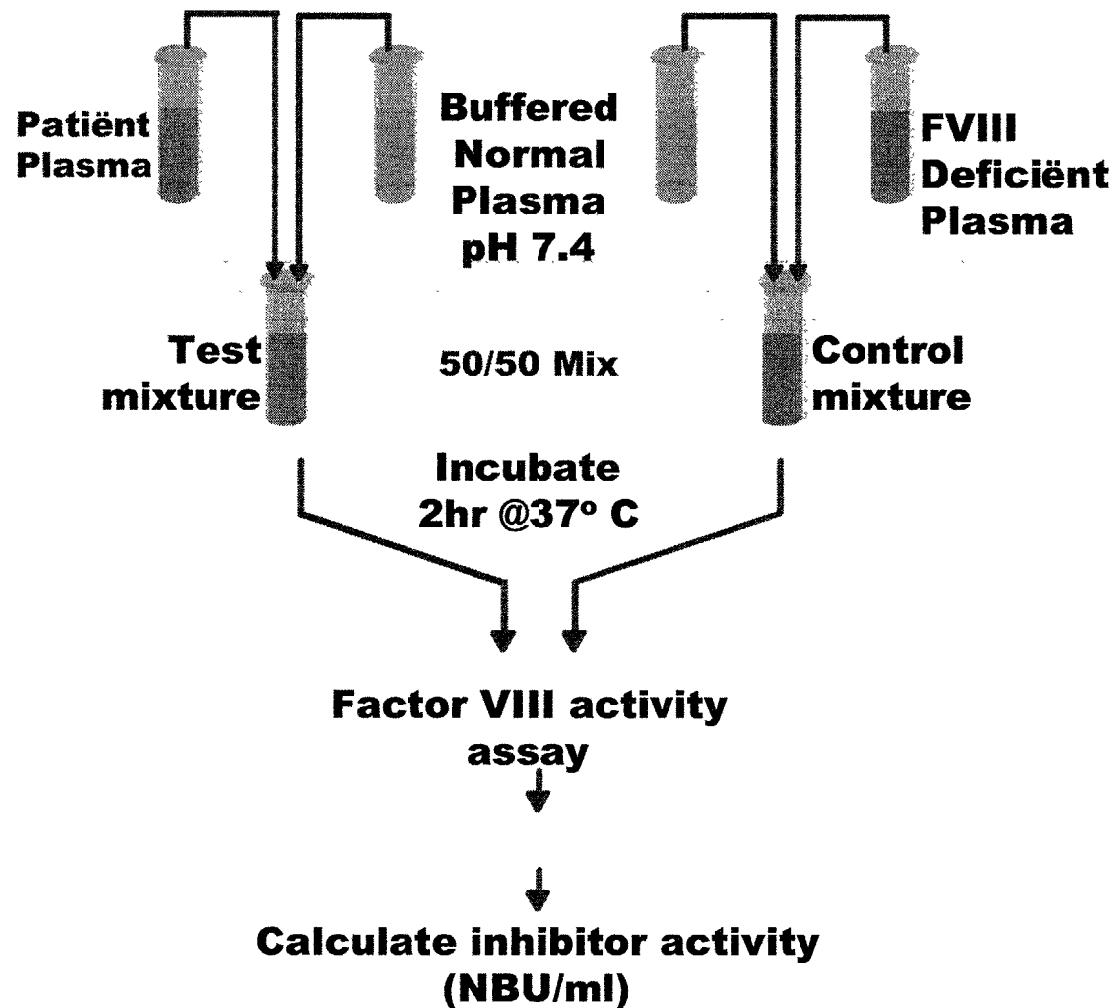
Sensitivity (3)

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Sensitivity (3)

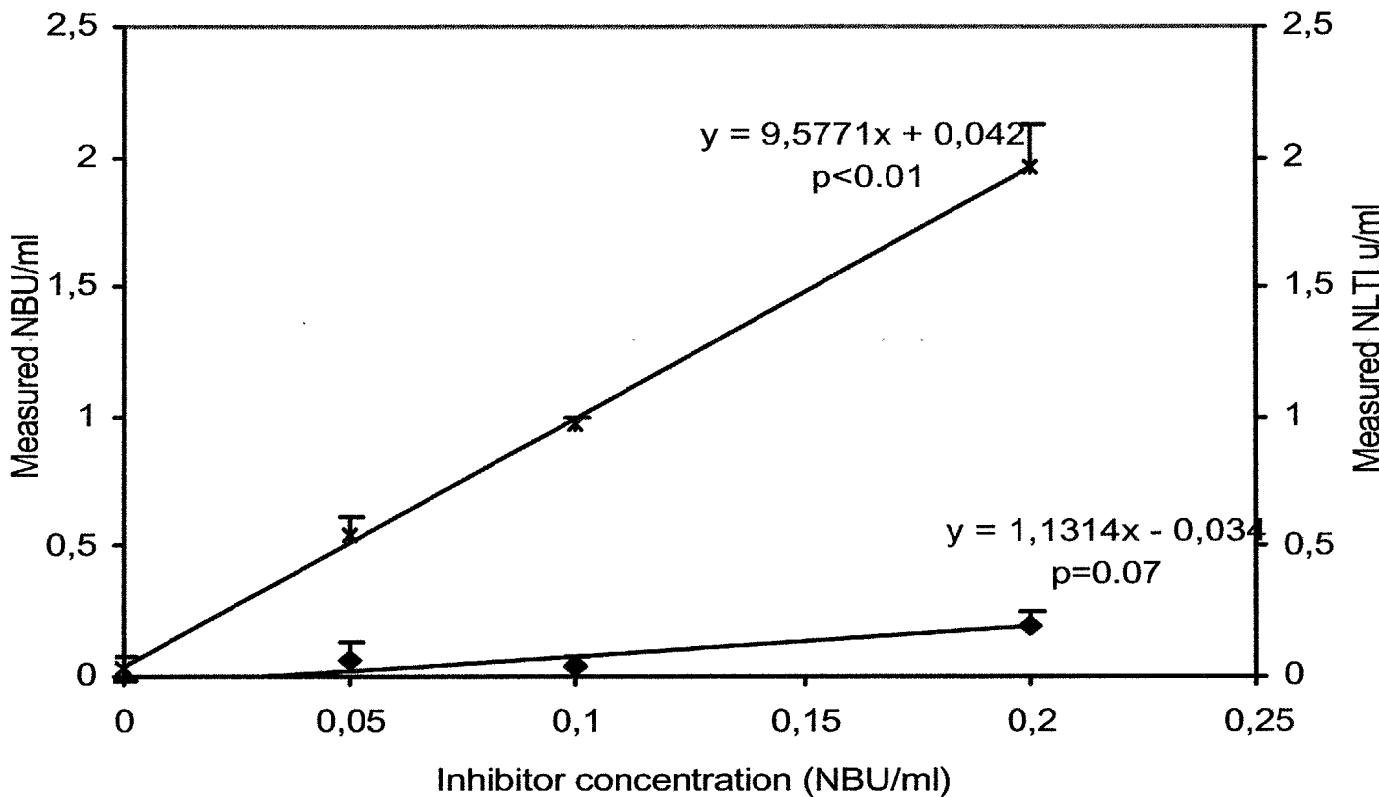
- F.VIII kinetics after replacement and bleeding status are “golden standards” but not “18 carat”
- The cutoff value for the Nijmegen Bethesda assay is 0,4 NBU/ml, however not evidence based and only slightly better than in the Classical Bethesda assay
- The sensitivity of inhibitor assays depends on the ability to detect small changes in F.VIII:C activity in a testmixture but coagulation assays only have limited precision to detect small differences in F.VIII activity

NIJMEGEN-BETHESDA ASSAY



Nijmegen Low Titre Inhibitor Assay

1. Concentration of plasma (patient and control) by selective protein filtration by centrifuge technique (concentration rate ca. 4 times)
2. Residual F.VIII in patient plasma is destroyed with EDTA
3. Assay of inhibitor activity by mixing with normal plasma pool in 1:3 ratio



Titres of inhibitor-free plasmas of haemophiliacs (n=7): 0.1 NLTI units /ml (sd 0.1)

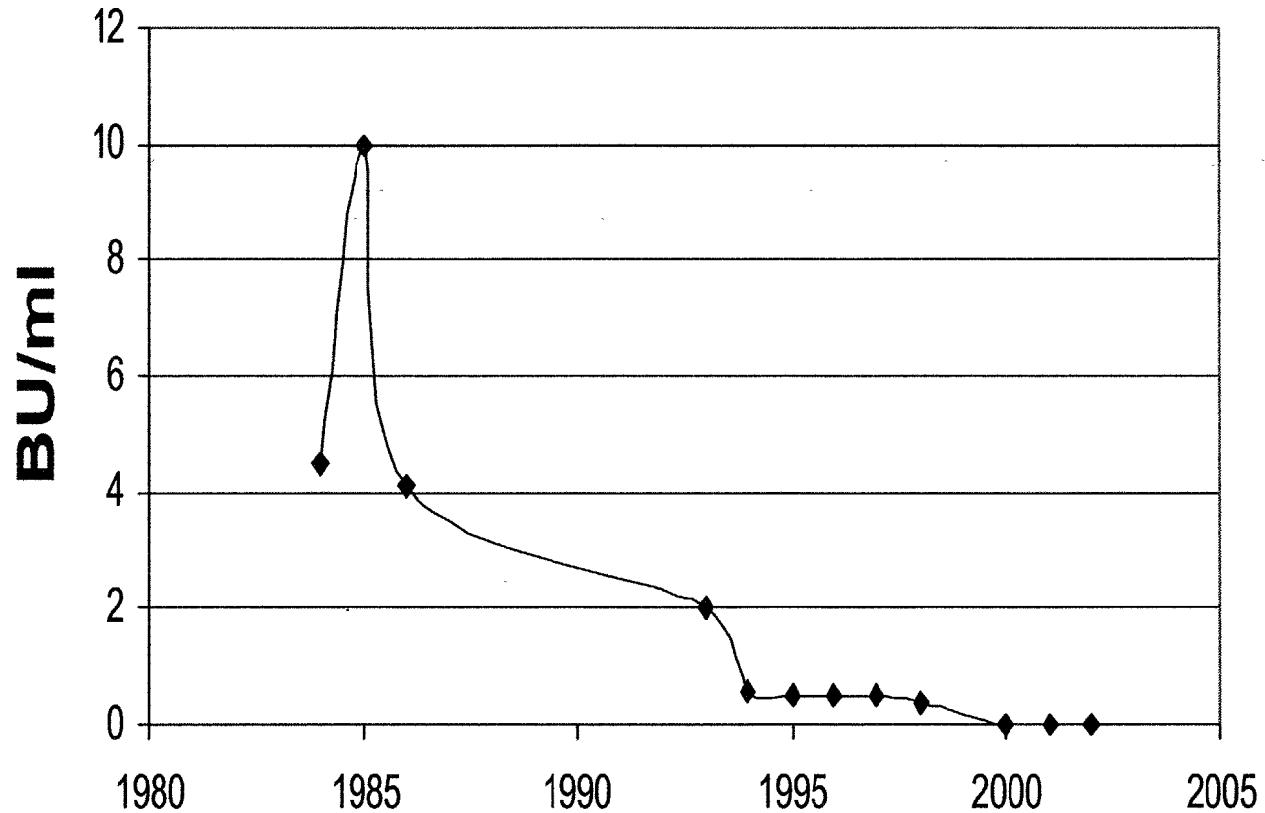
The Nijmegen Low Titre Inhibitor
Assay has a detection limit that is
10-15 times lower than the Nijmegen-
Bethesda assay.

Should Low Titres Be Of Concern?

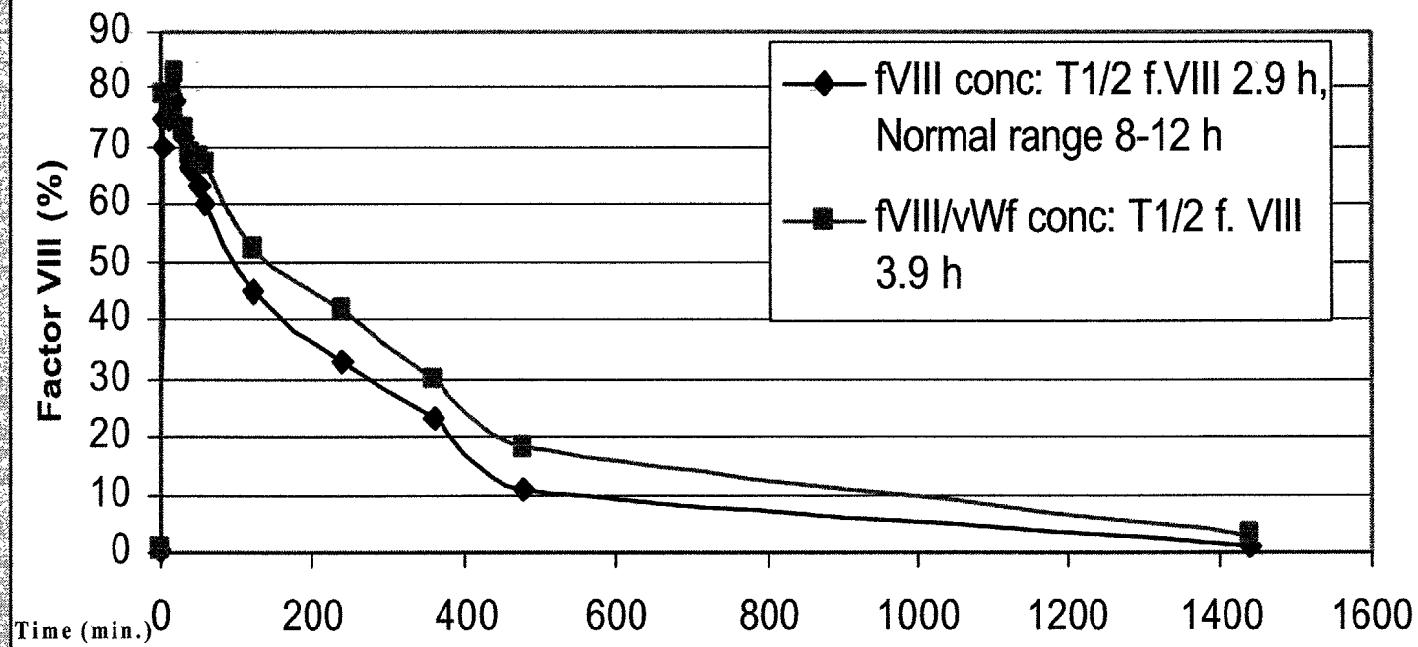
Case 1

- Man, born in 1972
- Haemophilia A
- Phenotype: factor VIII <1%, vWF 40%
- Genotype: Inversion intron 1

Case 1:Factor VIII inhibitor titre



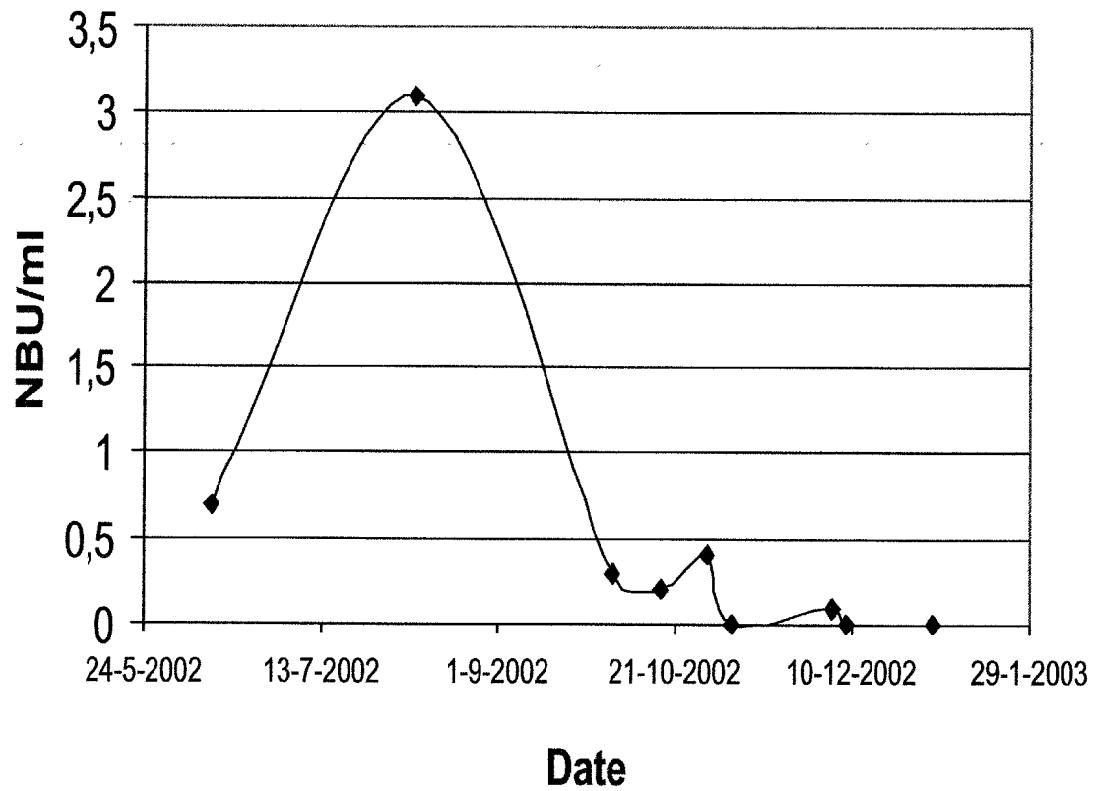
Case 1: Single-dose (25 IU/kg) decay curve of factor VIII



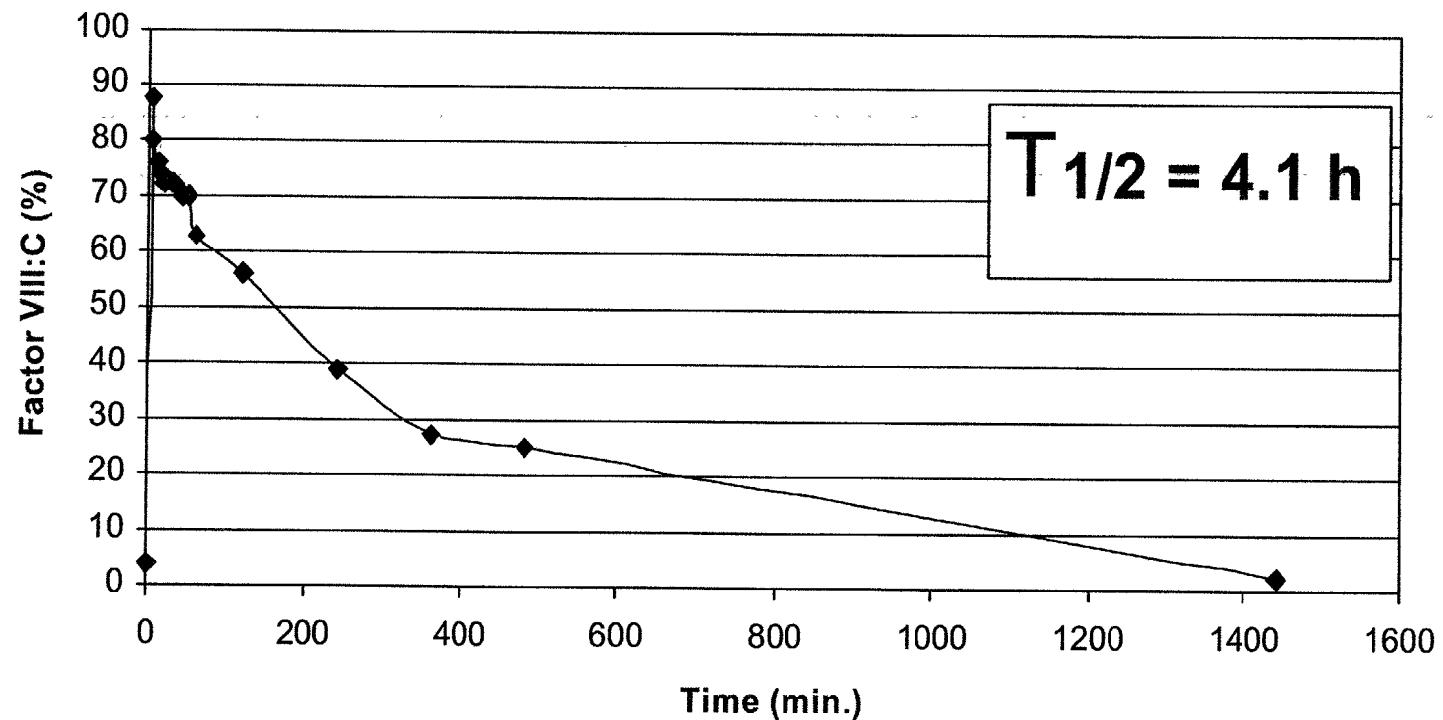
Case 2

- Man 61, born in 1942
- Haemophilia A
- Phenotype: factor VIII <1%, vWF >100%
- Genotype: Inversion intron 22

Case 2: Factor VIII inhibitor titre



Case 2: Single-dose (25 IU/kg) decay curve of FVIII



- Clinical presentation in both patients suggests presence of the inhibitor
 - Unusual bleeding tendency
 - Fast disappearance of factor VIII
- Nijmegen-Bethesda assay was negative

Case 1: Factor VIII:C inhibitor

Date	Inhibitor titre (NBU/ml)	Nijmegen Low Titre Inhibition Assay (NLTIA U/ml)
10-1993	2.0	
10-1994	0.6	
04-1998	0.4	7.03
04-2002	0.0	1.38
10-2003	0.0	0.32

Case 2: Factor VIII:C inhibitor

Date	Inhibitor titre (NBU/ml)	Nijmegen Low Titre Inhibition Assay (NLTIA U/ml)
6-2002	0.7	
8-2002	3.2	
10-2002	0.3	
11-2002	0.0	1.2
1-2003	0.0	1.5
10-2003	0.0	0.0

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Low titers of Factor VIII:C inhibitors may be of clinical relevance.

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More data have to be gathered to get more evidence about this problem.

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